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**FACSIMILE COVER LETTER**

**To:** Examiner: Shin Lin Chen Group: 1632  
**Firm:** U.S.P.T.O.  
**Facsimile:** 571-273-0726  
**From:** Marilyn Matthes Brogan  
**Date:** February 24, 2005  
**Re:** Applicant: Loerz et al  
Application No.: 09/674,824  
Filed: November 6, 2000  
For: NUCLEIC ACID MOLECULES WHICH CODE FOR ENZYMES  
DERIVED FROM WHEAT AND WHICH ARE INVOLVED IN THE  
SYNTHESIS OF STARCH  
Our Ref. No. 514413-3848

**Number of Pages:** 2  
(including cover page)

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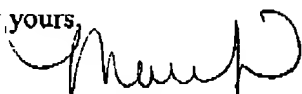
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Dear Mr. Chen:

Further to our earlier conversation of today, attached is a copy of page 30 of the specifications to the above referenced matter.

Best regards.

Very truly yours,



Marilyn Matthes Brogan

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**CONFIDENTIALITY NOTICE**

The documents accompanying this transmission contain confidential information intended for a specific individual and purpose. The information is private, and is legally protected by law. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution, or the taking of any action in reliance on the contents of this facsimile is strictly prohibited.

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WO 99/58688

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PCT/EP99/03156

The method for transforming immature wheat embryos was developed and optimized by Becker and Lörz (D. Becker and H. Lörz, Plant Tissue Culture Manual (1996), B12: 1 to 20).

- 5 In the experiments described hereinbelow, the procedure developed by Becker and Lörz (loc. cit.) was adhered to.

10 For the transformation, ears with caryopses of developmental stage 12 to 14 days after anthesis were harvested and surface-sterilized. The isolated scutella were plated onto induction medium #30 with the embryo axis orientated towards the medium.

15 After preculture for 2 to 4 days (26°C, in darkness), the explants are transferred to medium #39 for the osmotic preculture (2 to 4 h, 26°C, in the dark).

20 For the biolistic transformation, approx. 29 µg of gold particles onto which a few µg of the target DNA had previously been precipitated were employed per shot. Since the experiments carried out are cotransformations, the target DNA added to the precipitation batch is composed of the target gene and a resistance marker gene (bar gene) in the ratio 1:1.

#### 4. DIG labeling of DNA fragments

- 25 DNA fragments employed as screening probes were labeled via a specific PCR with the incorporation of DIG-labeled dUTP (Boehringer Mannheim, Germany).

Media solutions used in the examples:

30

20 × SSC                      175.3 g NaCl  
                                    88.2 g sodium citrate  
                                    twice-distilled H<sub>2</sub>O to 1000 ml  
                                    10 N NaOH to pH 7.0

- 35 Plasmid pTaSSI 8/1 was deposited at the DSMZ in Braunschweig, Federal Republic of Germany, as specified in the Budapest Treaty under the No. DSM 12794.

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page 30 for the missing  
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Plasmid pTaSSI  
Republic of Ger